2 Materials and Methods

Test Description

Wave-current (2-D) test flume

Tests were conducted in a 64-m-long by 1.5-m-wide concrete flume with wave and current generating capabilities (Figure 2). The flume (Figure 2a) is 2.0 m deep at the wave generator, from where the bottom rises at a 1:44 (V:H) slope to the test-section depth of 1.5 m. The test section extends to 28.7 m in length, and culminates into a rock wave absorber set at a slope of 1:6. In addition, rubberized matting ("horsehair") was placed at the far end of the test channel to minimize wave reflection back onto the test plants by absorbing wave energy. A 14.6-m-long glass observation area is built into one side of the flume test section.

Monochromatic wave trains were produced within the flume by an electro-hydraulic piston-type wave generator controlled by a computer-generated signal using software developed at WES. Currents were produced by a Gould Model 3410 electric pump plumbed with a 25.4-cm-diam intake pipe of polyvinyl chloride (PVC) and a 20.3-cm-diam PVC exhaust pipe. Plumbing to and from the pump to the flume consisted of a 25.4-cm-diam PVC pipe positioned alongside the flume at floor level. Flume inflow/outflow was routed first through a floor pit at either end of the flume and then through a 20.3-cm-diam PVC pipe that passed through the flume sidewall. These sidewall pipes were connected in series to the larger diameter pipe running alongside the flume to the pump to complete the closed circuit.

To obtain the desired water depth of 0.5 m in the flume, while simultaneously maintaining maximal hydraulic head for reaching higher current velocities, a false bottom was built within the test section of the flume (Figure 2a). Current within the flume is typically adjusted to desired velocities by valves in the large pipe running alongside the flume wall. For this set of tests, however, desired current velocities could not be obtained within the full 1.5-m width of the flume, even though the depth had been effectively reduced by inclusion of the false bottom in

the test section. To reduce volume, and thereby increase attained velocities, removeable divider walls were constructed of 3.8-cm-thick plywood that, when positioned, reduced the operating flume width to either 0.76 m or 0.38 m. The divider wall was 1.22 m high by 17.1 m long (Figure 2a).

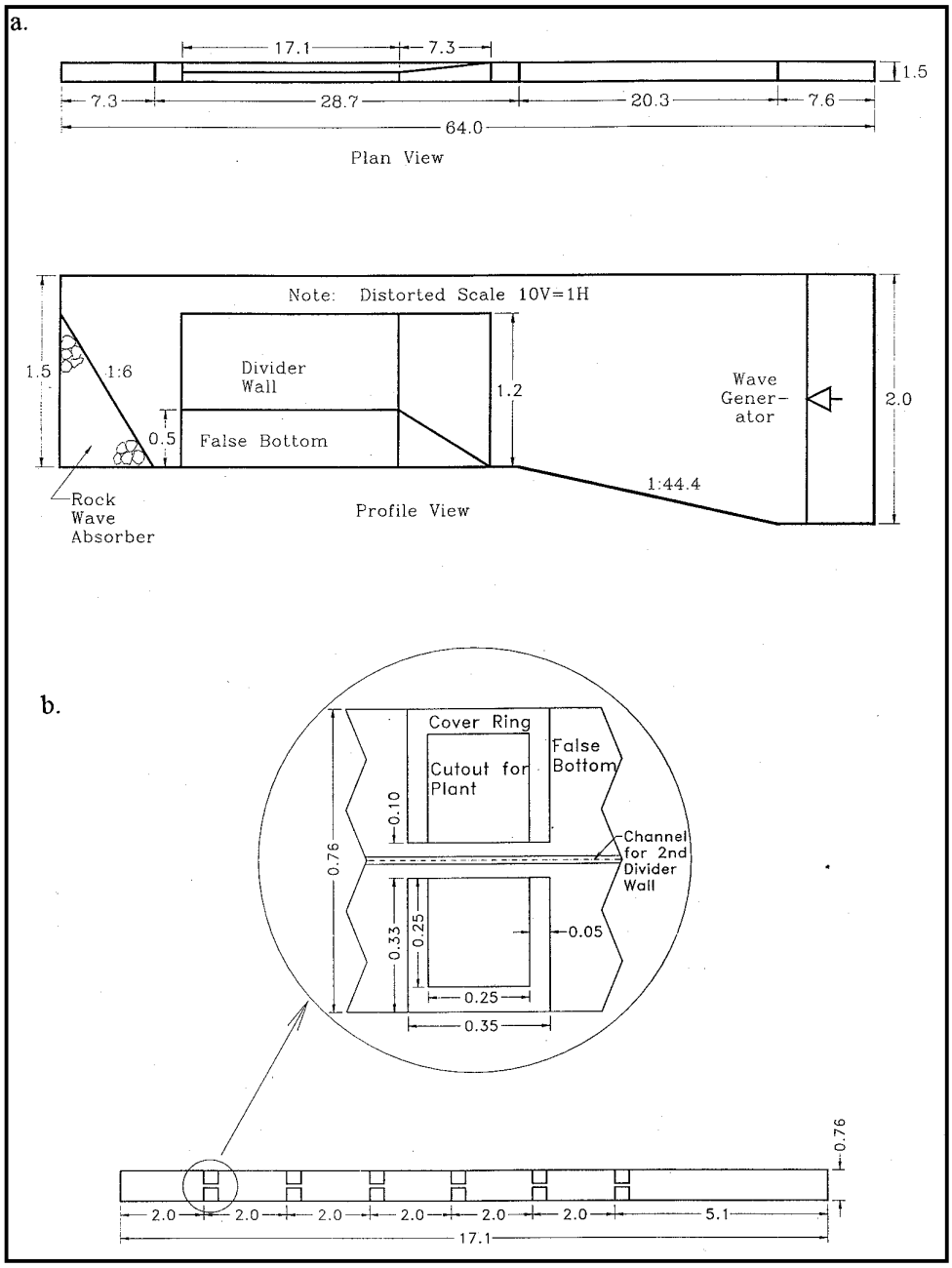

Figure 2. Schematic of flume facility: (a) plan and profile views of the flume structure with secondary walls and false bottom, (b) plan view with enlarged cutout showing positioning and construction of cutouts for placement of plant flats during treatment runs

Twelve rectangular holes approximately 35 cm by 33 cm were cut out of the false bottom for placement of test plant containers (Figure 2b). The size of the holes was such that a test plant container could be positioned flush with the flume false bottom and touching one side of the rectangular hole. The top rims of the test plant containers were then locked in place by fastening a C-shaped plywood ring around the other three sides of the containers (Figure 2b). The twelve holes were positioned in pairs, with 2 m between pairs. A wire screen made of 1.27-cm hardware cloth was positioned behind each pair of holes. Since containers of the same species were never assigned as pairs during a test run, fragments collected on the screens could be sorted by species and properly enumerated.

Wave period and height

The range of wave heights (0.1, 0.2, and 0.3 m) and wave periods (3 and 5 sec) used in this investigation were based on the range of typical wave heights and periods reported for navigation-generated secondary waves in the UMR system (see Chapter 1). Wave settings were monitored by capacitance-type wave gauges located in a three-gauge array directly in front of the wave maker (i.e., gauge number 1-3) plus three gauges (i.e., gauge numbers 4-6) spaced along the 17.1-m test section. Wave heights were calculated using software developed by WES. Data collected by gauges 1-3 were used to ensure repeatability of the wave signals, whereas gauges 4-6 monitored wave conditions affecting the test plants. Wave gauges were removed prior to initiation of each test run to prevent entanglement of plant material on the wave gauge rods.

Wave heights used in this study were $H_{1/3}$, or the average height of the one-third highest wave heights in a time series (also called H_s or significant wave height). During preliminary testing, $H_{1/3}$ and $H_{1/10}$ (average of the one-tenth highest wave heights in a time series) waves generated within the flume were nearly identical. Waves in each test were generated as purely monochromatic (i.e., equal period) wave trains with uniform wave height. However, flume effects, such as sidewall reflectance of wave energy, end flume effects, and shoaling, caused some disruption of the wave train.

Wave-height calibration data were collected in preliminary tests by determining the required wave generator stroke amplitude (cm) needed to create the desired wave heights, as measured by wave gauges 4-6 within the 17.1-m test section of the flume. The wave generator was calibrated prior to each treatment run for that treatment's unique combination of current, wave period, wave height, and operating flume width, which was narrowed from 0.76 m to 0.38 m for high-current velocity treatments. Calibration tests were run for 300 sec for each wave generator stroke setting. Calibration was generally based on recorded output between 60 and 120 sec of the test run. This ensured that the generated wave train had time to reach maximum heights within the test flume section and allowed evaluation of the impacts of end flume reflectance on the wave train over time.

Current velocity

The range of current velocities used in this investigation was selected to evaluate whether current velocities typical of ambient flows along the UMR main channel border can affect the direct effects of navigation-generated secondary waves on submersed plants. Though higher ambient currents sometimes occur along the main channel border, these higher flows could not be generated in the test flume as configured for this study.

Current velocities were measured by a Sonntec acoustic-doppler velocimeter (ADV) at the test section head. Signals from the ADV were displayed directly on a computer monitor using proprietary software from Sonntec. Currents used in this investigation were 0.0 m/sec (no current), 0.10 m/sec, and 0.25 m/sec. To establish the current, the circulating pump was started and allowed to run until the velocity had stabilized. Valves at each end of the circulation pipe allowed velocity control. After the desired current was reached and stabilized, the ADV was removed prior to test wave train initiation to prevent inundation of the meter by the wave action.

Drag measurements

A load cell previously developed by the WES Instrumentation Services Division was made available for measurements of tensile loading stemming from "drag" on plant shoots induced by waves during this study. The load cell had a minimum resolution of approximately 5 g. One end of the load cell and its attached wiring were fastened to the wooden bottom within the flume test section. The attachment was such that allowed the load cell to pivot at an angle projecting along the length of the flume. The wiring from the load cell was attached and run along the interior flume wall so that it exited from the top, from where it was routed to electronic signal processing equipment. The free end of the load cell was fitted with a piece of 6.35-mm-diam surgical tubing approximately 3 cm in length. The bases of intact plant shoots were attached to the surgical tubing. Under conditions of no current or waves, the plant shoot buoyancy was such that it lifted the plant shoot and load cell vertically into an upright position. The pivoting action of the load cell attachment allowed the plant shoot and load cell to maintain orientation in line with either drag forces generated on the plant shoot's base current or passing wave.

Plant Species Selection

This study focuses on Eurasian watermilfoil and vallisneria, two common species of submersed macrophytes in the UMR system. These particular species were selected for study because of their ecological significance to the UMR and because of distinct differences in their growth forms.

Vallisneria is a favorable native species with long, ribbon-like leaves that arise from a basal rosette (Haller and Sutton 1975; Fassett 1975; Godfrey and Wooten 1979). This species grows well at 20 to 32 ^{o}C (Barko, Hardin, and Matthews 1982) and can achieve lengths of 2 m or more depending on water depth (Korschgen and Green 1988). Since vallisneria does not produce a canopy, it typically does not interfere with the use of water resources. Nearly all parts of the plant, especially tubers and rootstocks, are eaten by a variety of aquatic animals and migratory wildfowl (Haller 1974; Korschgen, George, and Green 1988), and its leaves provide habitat and shelter for communities of invertebrates and spawning sport fish (Muencher 1944; Haller 1974; Poe et al. 1986). Established colonies of vallisneria help to improve water quality by filtering out suspended matter, stabilizing sediments, and reducing nutrient concentrations that would otherwise promote algal growth (Korschgen and Green 1988; Korschgen 1990; Barko, Gunnison, and Carpenter 1991; Smart, Barko, and McFarland 1994).

In contrast, Eurasian watermilfoil is an exotic perennial with finely dissected leaves and long, flexible stems (Grace and Wetzel 1978). This species grows rapidly at temperatures from 16 to 35 ^{o}C (Barko and Smart 1981; Smith and Barko 1990), and in a single growing season can achieve lengths in excess of 4 m (Grace and Wetzel 1978; Eggers and Reed 1987). Roots of this species are adventitious, forming on upper portions of the stem prior to autofragmentation and on lower stems buried in sediment (Shannon 1953; Grace and Wetzel 1978; Smith, Barko, and McFarland 1991). As the plant grows, biomass is distributed at or near the water surface, forming a dense mat or canopy of entangled stems and branches. Self-imposed shading beneath the canopy causes a loss of lower leaves on older plants (Adams, Titus, and McCracken 1974). Stolons expand the population locally over a few meters; however, fragments are the predominant means of long distance dispersal and colonization (Kimbel 1982; Madsen, Eichler, and Boylen 1988). Excessive growth of milfoil can be problematic due to the crowding out of native vegetation, and negative impacts on water quality, recreational use, fish and wildlife habitat, and aesthetics (Smith and Barko 1990).

For each species, both 4-week- and 8-week-old plants were tested to provide intraspecific differences in morphology due to developmental stage. From previous experience at WES with greenhouse plant cultures, 8-week-old plants were expected to possess greater biomass, length, and shoot density (i.e., number of shoots per flat) than the 4-week-old plants. In addition, the onset of senescence evidenced by flower and fruit production was expected to occur after 8 weeks in culture. A detailed description of sexual reproduction in milfoil is provided in Grace and Wetzel (1978), and in vallisneria in Kaul (1970).

Plant Culture Techniques

To furnish the large numbers of plants required for flume exposures, an intensive planting effort was initiated in the spring of 1995. Monocultures of vallisneria and milfoil were grown in 1,200-L white fiberglass tanks housed in a greenhouse facility at WES. Tanks were filled 83 cm deep with the low alkalinity culture solution described in Smart and Barko (1985). This solution, prepared

with reagent-grade salts and deionized-distilled water, provides major cations (Na^{+} = 16.0, K^{+} = 6.0, Ca^{+2} = 25.0, and Mg^{+2} = 6.8 mg/L) and anions (Cl^{-1} = 44.2, HCO_3^{-} = 51.8, and SO_4^{-2} = 26.9 mg/L) but lacks N and P, specifically omitted to minimize algal growth and associated light reductions in the water column. Upon preparation, the solution had a pH of 7.9 and an electrical conductivity of 278 microsiemens/cm (µS/cm). Two air lifts per tank provided filtered-humidified air to enhance air/water CO_2 exchange. Solution temperatures were maintained at 25° C (± 1 °C) using Remcor circulators plumbed singly to each tank. Temperatures were monitored 2 or 3 times per day with minor thermostat adjustments made as necessary.

Surficial sediment dredged from Brown's Lake at WES provided the rooting medium for the cultures. Sediment from this lake, collected from a site devoid of aquatic vegetation, has been used in WES laboratories for many years to culture a variety of submersed aquatic plants. This fine-textured, inorganic sediment (characterized in McFarland and Barko 1987) has particle size fractions of ≈ 10 percent coarse (> 50 µ diam) and ≈ 90 percent fine (< 50 µ diam) by dry mass. The sediment was amended with ammonium chloride (0.8 g per L wet sediment) while mixing thoroughly in a large-capacity mortar mixer. This chemical amendment was provided to ensure sufficient nitrogen availability for 8 weeks of growth. When mixing was completed, the sediment was poured to a depth of 8 cm in 24.3- by 24.3- by 10.0-cm polyethylene containers. The sediment was then allowed to settle at least two weeks prior to planting. A summary of physical and chemical characteristics of the sediment (after fertilization) as determined by analytical procedures described in Barko et al. (1988) is in Appendix A.

All plants were grown at ≈ 25 percent full sunlight using neutral density shade fabric draped over the greenhouse roof. Maximum midday photosynthetically active radiation (PAR) levels inside the tanks reached ≈ 400 µE/m^2/sec. At this location (i.e., Vicksburg, MS; 32°23'N, 90°52'W), the duration of daylight ranged from 13.4 to 14.3 hr between late April and late July (List 1951).

Eurasian watermilfoil used in the study was clipped 15 cm in length from apices of a continuous WES greenhouse stock. This stock was established from a previous collection in Lake Wingra, WI. Overwintered tubers of vallisneria were obtained commercially from a wildlife nursery in Oshkosh, WI and were sorted prior to planting to ensure size uniformity.

Each species was planted separately at a density of 9 propagules per flat (24.3 by 24.3 by 10 cm deep). The propagules were spaced evenly in the containers, with basal ends of vallisneria buried ≈ 2 cm and milfoil ≈ 4 cm deep in sediment. A thin layer of washed silica sand was placed over the sediment surface to prevent physical mixing with the overlying solution. Immediately after planting, the containers were submersed into prepared culture tanks.

One tank per species was planted each week from late April to late June 1995 (Table 1). Weekly plantings were required to provide plants of two age groups

Table 1
Planting Schedule for Culture Plants

Month	April				May				June				July				August			
Week	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Obtain Propagules			*	*																
M8-1 & V8-1 Culture				P	*	*	*	*	*	*	*	T								
M4-1 & V4-1 Culture								P	*	*	*	T								
M8-2 & V8-2 Culture					P	*	*	*	*	*	*	*	T							
M4-2 & V4-2 Culture									P	*	*	*	T							
M8-3 & V8-3 Culture						P	*	*	*	*	*	*	*	T						
M4-3 & V4-3 Culture										P	*	*	*	T						
M8-4 & V8-4 Culture							P	*	*	*	*	*	*	*	T					
M4-4 & V4-4 Culture											P	*	*	*	T					
M8-5 & V8-5 Culture								P	*	*	*	*	*	*	*	T				
M4-5 & V4-5 Culture												P	*	*	*	T				
M8-6 & V8-6 Culture									P	*	*	*	*	*	*	*	T			

(i.e., 4 and 8 weeks) for weekly exposures for one month (July) in the flume. Seven flats were positioned in each culture tank, allowing six flats for flume exposures and one for pretreatment assessments of biomass, morphology, and tensile strength.

Pretreatment Plant Measurements

Milfoil measurements

After attaining their preselected age in culture, the growth characteristics of the dominant shoots originating from 15 plants from each culture tank were measured. For this, dominant shoots which had been produced by all nine of the original apical plantings of one of the seven culture flats were removed for pretreatment measurements. Additionally, the dominant shoot originating from one of the nine original apical tips was removed from each of the remaining six culture flats, resulting in a total of fifteen shoots for pretreatment measurements. These measurements included length (mm) along main shoot axis, total number of meristems (including all branches), and dominant shoot biomass. Additionally, the 15 dominant shoots were divided into three zones for characterization of mechanical properties. Zone 1 included the portion of the shoot from its base to 40 cm distally. Zone 2 included an indeterminate length of the main shoot between Zone 1 and Zone 3, which consisted of the 40-cm section of the shoot from its apex toward the base. For each of the three zones, the proximal end of each shoot section was attached to a customized gripping fixture attached to a LICOR Model DFGS-10 digital force gauge. The force gauge was mounted horizontally on a table, with the display facing upwards. The shoot section was grasped distally by hand and slowly pulled along a graduated (cm) line originating from the force gauge gripping fixture until breaking. The breakage force (N) was recorded, and the diameter of the shoot section at its breaking point was measured (mm) with a Mitutoya Model CD-6"BS digimatic caliper.

Tensile strength estimates for each shoot section were calculated using the equation:

$$T = B/A,$$

where

T = tensile strength (N/mm^2)
B = breakage force (N)
A = cross-sectional area of the shoot at the breaking point (mm^2).

After all 15 dominant shoots were measured, all shoot material from one flat for each culture tank was dried and weighed for later calculation of mean flat shoot biomass per age group.

Vallisneria measurements

Pretreatment physical measurements were also made for 15 plants in the vallisneria culture tanks. As for milfoil, these measurements were made for primary shoots growing out of the original propagule. For vallisneria, the original propagule was a tuber, and the primary shoot was an entire plant consisting of a basal rosette and numerous leaves. Depending on the plant age (i.e., 4 or 8 weeks), flower pedicels and ramets had also been produced. Plants were removed intact from the culture flats. Roots were clipped at their attachment point to the main rhizome, as were any attached ramets. The plant was then attached basally by the naked root crown to the force gauge, and the leaves and flower pedicels were extended distally along the graduated scale. Physical measurements for each plant included length of longest leaf (cm), number of leaves, number of flower pedicels, length (cm) of flower pedicels, force (N) to break flower pedicels by pulling distally, and diameter (mm) of flower pedicel at breaking point. Tensile strength measurements for the flower pedicels were estimated using the same calculations as described above for milfoil. Tensile strength estimates were not calculated for vallisneria leaves, due to inaccuracy in estimating leaf cross-sectional dimensions (i.e., especially thickness). As for milfoil, estimations were made of the mean shoot biomass of culture flats for the two age groups of plants.

Study Design and Execution

Hydrological treatment combinations

The study incorporated the establishment of 18 treatments (Table 2) based on different combinations of current velocity, wave periods, and wave heights. Plants were exposed to each treatment for 25 min. Each test run (i.e., SPECIES by AGE by TREATMENT) included three replications (n = 3), with each replication consisting of a flat of plants resulting from the growth (i.e., over 4 or 8 weeks) of 9 original apical tips. Pretreatment culture techniques for the test plants are described above.

Test setup and treatment sequencing

On their selected treatment day, planted flats were removed from the culture tank and placed individually into ice chests fitted with a customized foam rubber bottom fashioned to keep the flat stationary during transport. At the flume facility, the flats were removed from the ice chests and placed in the rectangular cutouts in the flume bottom (Figure 2b). Because some entanglement and breakage was unavoidable during flat transport and placement into the flume, plant shoots/leaves were untangled, and broken fragments were removed by gentle hand-teasing under a low velocity current (<0.1 m/sec). This procedure also resulted in the plants being oriented with the current and wave direction prior to treatment initiation. Any fragments coming off the plants during this setup phase were collected and placed in a labeled bag.

Table 2
Hydrological Conditions and Culture Tank Sources of Test Plants for Each Treatment

Treatment ID	Current Velocity, m/sec	Wave Period, sec	Wave Height, m	Plant Source/Culture Tank[1]			
				Milfoil		Vallisneria	
				4 wk	8 wk	4 wk	8 wk
1	0.25	3	0.1	M4-2	M8-2	V4-2	V8-2
2	0.25	3	0.2	M4-2	M8-2	V4-2	V8-2
3	0.25	3	0.3	M4-2	M8-2	V4-2	V8-2
4	0.25	5	0.1	M4-3	M8-3	V4-3	V8-3
5	0.25	5	0.2	M4-3	M8-3	V4-3	V8-3
6	0.25	5	0.3	M4-3	M8-3	V4-3	V8-3
7	0.10	5	0.1	M4-4	M8-4	V4-4	V8-4
8	0.10	5	0.2	M4-4	M8-4	V4-4	V8-4
9	0.10	5	0.3	M4-4	M8-4	V4-4	V8-4
10	0.10	3	0.1	NR[2]	M8-6	NR[2]	V8-6
11	0.10	3	0.2	NR[2]	M8-6	NR[2]	V8-6
12	0.10	3	0.3	NR[2]	M8-6	NR[2]	V8-6
13	0.00	3	0.1	M4-5	M8-5	V4-5	V8-5
14	0.00	3	0.2	M4-5	M8-5	V4-5	V8-5
15	0.00	3	0.3	M4-5	M8-5	V4-5	V8-5
16	0.00	5	0.1	M4-5	M8-5	V4-5	V8-5
17	0.00	5	0.2	M4-5	M8-5	V4-5	V8-5
18	0.00	5	0.3	M4-5	M8-5	V4-5	V8-5

[1] Culture tank is the number after the hyphen.
[2] Not run. Tests were not conducted for 4-week-old plant exposure to Treatment 10, 11, or 12.

Tests were initiated by first establishing the desired current velocity in the flume. For a given current velocity and wave period, three treatment runs were then conducted resulting in sequential exposure of plant flats (n = 3 for each combination of species by age) to the three wave heights (i.e., 0.1, 0.2, and 0.3 m) (Table 2). Each of the wave height exposures was 25 min, after which time wave propagation was terminated, and fragments were collected from the screens before initiation of the next wave height exposure in the series. After collection of fragments from the last wave height exposure (i.e., 0.3 m) in the series, plant flats were removed from the flume. All remaining aboveground plant material was cut at the sediment surface and placed in a labeled bag, along with any plant fragments that had been collected during the test-run setup phase.

Treatment effects measurements

Fragments collected during exposure of a given plant species and age to a treatment (i.e., current velocity by wave period by wave height) were placed into a tray of water. Fragments were counted, characterized as to type (i.e., flower, apical or non-apical stem section (milfoil), or leaf section (vallisneria)), fragment length, fragment diameter at breaking point (milfoil stem or vallisneria pedicel), number of nodes (milfoil stem), and number of meristems (milfoil stem). After enumeration, all fragments for a given treatment replication were placed in a labeled bag for determination of total fragment dry weight.

Data Analysis

Pretreatment measurements

Pretreatment plant measurements were analyzed both by culture tank and by age for a given species. For a given species and age, a one-way analysis of variance (ANOVA) using a general linear models (GLM) procedure was conducted to determine significant differences ($p = 0.05$) in shoot growth between culture tanks, with mean separations ($n = 15$) being provided by a Fisher's Least Significant Difference (LSD) test (SAS 1988). A separate ANOVA was also conducted by age group ($n = 60$ shoots) for each species to determine which parameters were significantly different ($p = 0.05$) between age groups.

Treatment effects comparisons

For both plant species and age groups, fragment damage resulting from each of the test treatments was analyzed by a one-way ANOVA (PROC GLM, SAS 1988). Means separations for the amount of damage resulting from the different treatments were performed using Fischer's LSD test ($p = 0.05$). Parameters used to assess fragment damage were cumulative fragment number and cumulative fragment dry weight per treatment. Based on assumptions of the experimental design, these cumulative values were derived by summing fragment collections across sequential wave height exposures. As an example, and referring to Table 2, analysis of Eurasian watermilfoil exposure to Treatments 1-3 were conducted by comparing fragments from Treatment 1 to the cumulative fragments collected through Treatment 2 and to the cumulative fragments collected through Treatment 3. These summations were deemed necessary since all three wave height treatments for a given velocity and wave period combination were run in a series and were conducted using the same plants. Therefore, fragments broken by the 0.1-m wave treatment were not available for breakage by the 0.2-m wave treatment, and fragments broken by the 0.1- and 0.2-m wave treatment were not available during the 0.3-m treatment.

Mechanical Properties of Field Plants

In August 1995, plant collections were made from Lake Onalaska, WI, to provide tensile strength data of field-grown plants for comparison with tensile strength estimates of test plants used in this flume study. Field collections were made for Eurasian watermilfoil, sago pondweed (*Potamogeton pectinatus* L.), American pondweed (*P. nodosus* Poir.), curly leaf pondweed (*P. crispus* L.), Richardson's pondweed (*P. richardsonii* (A. Benn.) Rydb.), water stargrass (*Heteranthera dubia* Jacq.), and coontail (*Ceratophyllum demersum* L.). From these collections, the breaking force of the basal (Zone 1) and apical sections (Zone 3) of nine shoots of each species were measured. Stem diameters, or other dimensions of cross-sectional area, were measured at the breakage points for later tensile strength calculations.